

SCIENTIFIC NOTE

ALGAL FOOD SELECTION AND DIGESTION BY LARVAE OF THE PESTIFEROUS CHIRONOMID *CHIRONOMUS CRASSICAUDATUS* UNDER LABORATORY CONDITIONS

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ABSTRACT. Feeding preference of *Chironomus crassicaudatus* 4th instars when fed on 5 algal species, *Anabaena flos-aquae*, *Botryococcus braunii*, *Lyngbia* cf. *aeruginosa*, *Microcystis* sp., and *Scenedesmus quadricauda* was studied under laboratory conditions. The various algal species were mixed in pairs at 1:1 ratio (fresh weight) to create 10 possible test combinations. The larvae were allowed to feed individually for 8 h on each algal mixture in tissue culture plates having 4 replicates. Four identical algal mixtures were simultaneously used without larvae as controls. After feeding, larvae and excrement were removed, and remaining algae from feeding trials and controls were fixed with Lugol's solution; the final ratio of algal species in each mixture was determined microscopically. Feeding preferences of *C. crassicaudatus* early 4th instars, in descending order, was *L. cf. aeruginosa*, *A. flos-aquae*, *B. braunii*, *Microcystis* sp., and *S. quadricauda*. To evaluate algal digestibility, larval excrement was collected and the proportion of live and dead cells was determined by microscopic observations with the use of visible and ultraviolet light (epifluorescence). *Anabaena flos-aquae* and *L. cf. aeruginosa* were the easiest to digest, followed by *Microcystis* sp. and *S. quadricauda*, whereas no digestion of *B. braunii* was observed. Cultures of larval excrement revealed the presence of some viable cells of all 5 tested algal species.

KEY WORDS Chironomidae, *Chironomus crassicaudatus*, nuisance midge, cyanobacteria, Chlorophyta, food biology, digestion

Large numbers of adult midges that emerge from urban and suburban lakes in central Florida, USA, can cause severe nuisance and economic problems (Ali 1995, 1996). For the medium-sized city of Sanford, economic losses of \$3–4 million can occur annually to waterfront residents, visitors, and businesses (Anonymous 1977). *Chironomus crassicaudatus* Malloch (Diptera: Chironomidae) is one of the major nuisance chironomid species in central Florida (Ali 1995). Algae are the primary food component of larval *C. crassicaudatus* (Ali 1990) and some other chironomid species (Provost and Branch 1959); therefore, algae are an important factor that affects chironomid populations (Ali 1990, Ali et al. 2002). Many previous studies of chironomid larval food requirements and composition have been based on the larval gut contents (e.g., Provost and Branch 1959, Ali 1990, Frouz and Lukešová 1995) or excrement (Davies 1975). However, as pointed out by Frouz and Lukešová (1995), the interpretation of gut contents can depend upon digestibility of individual algal species; algae that are difficult to digest can remain undigested in the gut and excrement in higher proportions, compared with easily digestible algal species. Another problem that complicates estimation of food selectivity

in the field is the large variability of algal supply in the microhabitats occupied by midge larvae (Provost and Branch 1959). The goal of this study was to test whether *C. crassicaudatus* larvae feed non-selectively on individual algal species. The digestibility of these algal species by the larvae was also assessed. Five algal species (*Anabaena flos-aquae* Ralfs ex Born., *Botryococcus braunii* Kutz., *Lyngbia* cf. *aeruginosa* Agarth, *Microcystis* sp., and *Scenedesmus quadricauda* [Turp.] Breb.) were selected for the experiment. These species differ remarkably in size and shape but have been reported to predominate in *C. crassicaudatus* larval environments (Ali 1990). To assess food selectivity, we adopted a technique based on algal removal from the environment. This technique is a modification of a method used for the study of food selection preferences of filter-feeding crustaceans (Knisely and Geller 1986) and is not affected by variable algal digestibility.

For the study, *C. crassicaudatus* larvae were collected with an Ekman dredge from Lake Monroe, central Florida, during the last week of June 2003. A detailed description of the lake is available in Ali et al. (2002). The dredge samples were sieved through 0.35-mm mesh in the field and hand sorted in the laboratory to recover *C. crassicaudatus* early 4th instars; larval identification was confirmed with the use of Epler (2001) keys. The larvae were transferred to plastic dishes (20 × 30 cm, 5 cm deep) filled with 1× Martin's rearing solution (Martin et al. 1980) supplemented with thiamine 1.2 mg/liter

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(Stevens 1998). Autoclaved sand was added to each dish to prevent any larval cannibalism. The dishes were maintained under 22°C and 14:10 h light:dark. Preaerated rearing solution was replaced daily in the dishes. After field collection, the larvae were starved for a 48-h period prior to the algal feeding experiments.

Single-species cultures of *A. flos-aquae*, *B. braunii*, *L. cf. aeruginosa*, *Microcystis* sp., and *S. quadricauda* were obtained from the algal collection at the Department of Fisheries and Aquatic Sciences of the University of Florida, Gainesville. Cultures of these algal species were kept at 30°C under constant light in 1-liter Erlenmeyer flasks. Each culture was aerated continuously, and ~250 ml of suspension was harvested daily and replaced by the same volume of fresh culture media. Modified Allen medium (Allen 1968), buffered by HEPES and NaOH to pH 8.2, was used. As a nitrogen source, KNO₃ at 0.2 g/liter was added to the medium; however, this was omitted for the nitrogen-fixing species *A. flos-aquae*. The harvested algal suspension of each species was vacuum filtered through a 0.2- μ m membrane filter (Gelman Sciences, Ann Arbor, MI). Immediately after filtration, the membrane was removed from the funnel, and the filtered algae on top of the filter membrane were gently scraped into a beaker and weighed (model AS120, Ohaus Corp., Florham Park, NJ; accuracy 0.1 mg). The algae were then resuspended in 1 \times Martin's rearing solution at a concentration of 0.2 mg/ml (fresh weight). A magnetic stirrer (Corning, PG-353, Corning Inc., Corning, NY) and a 43-kHz ultrasound cleaner (1 min) were used for resuspension. In the case of *A. flos-aquae* and *L. cf. aeruginosa*, clumps were gently disrupted with a glass tissue grinder before resuspension. A 100-ml suspension of each algal species was thoroughly mixed in pairs to prepare 10 mixture combinations of the test algae. Four milliliters of each mixture suspension were added to each of 8 cells of a tissue culture plate (model Costar 3513, 12-well cluster, Corning) and incubated for 24 h. Preliminary observations revealed that clumped structures of *A. flos-aquae* and *L. cf. aeruginosa* reappeared after 24 h of incubation. One *C. crassicaudatus* early 4th instar was introduced into each of 4 cells containing each algal mixture, whereas 4 cells were kept without larvae to serve as controls. At 8 h after larval introduction, the larvae, excrement, and food bolus were removed. Algae in all 8 cells were fixed with Lugol's solution, covered, and stored in the dark at 10°C for up to 7 days. Before further processing, the fixed algae were resuspended by mechanical agitation and sonification for 3 min with the ultrasound cleaner. Two slides, each containing 35 μ l of suspension, were prepared from each cell of the tissue culture plate. The number of algal cells of individual species was counted with a square counting grid under a 400 \times magnification compound microscope (model BHA, Olympus Corp., Tokyo, Ja-

pan). A minimum of 50 fields were counted per slide. At least 400 fields for each experimental (with larvae) and each algal control combination were counted. The total number of algal cells counted in both control and experimental suspensions was kept equal. The proportion of cells of individual species in control and treatments (with larvae) was compared by χ^2 test, considering the counts in controls as an expected distribution.

To evaluate algal digestibility, *C. crassicaudatus* larvae were reared on individual algal species for 72 h, and excrement was removed daily. Excrement that was collected after 48 and 72 h was used to analyze the proportion of live and dead algal cells. This excrement was wet mounted on a microscopic slide and disrupted by gentle pressure on the cover glass to form a layer about 1 cell thick. Slides were observed under visible light at various magnifications with an Olympus BHA microscope and under ultraviolet (UV) light (epifluorescence) with a model MZFEIII microscope (Leica Microsystems Inc., Wetzlar, Germany). The cells that appeared green under visible light and red under UV light were assumed to be live, whereas brown or brownish cells under visible light that did not appear red under UV light were assumed to be dead. These were compared with similar slide mounts of membrane-filtered stock algal suspensions. Individual cells of *B. braunii* and *S. quadricauda* were easily distinguishable in the excrement, but for the other 3 algal species, the majority of the cells appeared as an amorphous mass with individual cells impossible to count. We assumed that the layer of dead and live cells on the slide was uniform in thickness on the whole slide, with 1 dead or live cell covering equal area. Hence, proportions of area covered by live or dead cells were assumed to be the proportion of live and dead cells on the slide. The area covered by live and dead cells in these masses was estimated with an ocular-mounted 10 \times 10 grid micrometer. At least 5 excrement and 5 filtered stock suspension samples were used for each algal species, and 10 fields were examined per sample. The proportion of live cells was calculated for each field and values were arcsine transformed to achieve normality. A 2-tailed Student's *t*-test was used to compare proportions of live cells in stock culture and in excrement produced for the same alga. One-way ANOVA was used to compare proportions of live cells among individual algal species.

To evaluate algal viability, 5 fecal pellets collected after 72 h of larval introduction were put in individual cells of a tissue culture plate described above and filled with 4 ml of appropriate rearing solution. After 1 h, excrement was transferred to a fresh culture cell with rearing solution; this practice was repeated twice to remove any algal cells attached to the excrement surface. The washed excrement was placed in fresh rearing solution and cultured for 7 days at 30°C under constant light. The number of excrement that produced new algal

Table 1. Final proportion (fresh weight) of algal species, combined in pairs, after 8 h of *Chironomus crassicaudatus* larval feeding under laboratory conditions. Proportion data format is row:column. Original proportion of all algal species pairs was a 50:50 ratio. Species of algae with a final proportion <50 were preferred by *C. crassicaudatus*. Significant differences (χ^2 test) from a 50:50 ratio are indicated by * $P < 0.05$ and *** $P < 0.001$.

Algal species	<i>Scenedesmus quadricauda</i>	<i>Botryococcus braunii</i>	<i>Microcystis</i> sp.	<i>Anabaena flos-aquae</i>
<i>S. quadricauda</i>				
<i>B. braunii</i>	20:80***			
<i>Microcystis</i> sp.	43:57*	82:18***		
<i>A. flos-aquae</i>	12:88***	32:68***	7:93***	
<i>Lyngbia</i> cf. <i>aeruginosa</i>	10:90***	15:85***	1:99***	19:81***

cultures of individual species was compared by the Kruskal-Wallis test.

In the food selection experiment (Table 1), significant differences in preference by individual algal species were noted; *L. cf. aeruginosa* was the most preferred, followed by *A. flos-aquae*, *B. braunii*, *Microcystis* sp., and *S. quadricauda*. This result is in agreement with the findings of Ali (1990), who reported that cyanobacteria was preferred over Chlorophyta by *C. crassicaudatus* larvae, with *Lyngbia* and *Anabaena* being present and *Microcystis* absent in the gut contents. This is contrary to the hypothesis that *C. crassicaudatus* larvae are nonselective feeders on algae. This type of food selection probably corresponds to shape and size of algae, which might interact with the size and shape of midge catch nets or larval mouthparts (Provost and Branch 1959, McLachlan et al. 1978). Filamentous cyanobacteria (*Lyngbia*) or those forming long chains of cells (*Anabaena*) might be trapped

more easily by the midge catch nets than the round-shaped *Botryococcus*. Similar mechanisms might explain the preference of larger sized *Botryococcus* over smaller sized *Microcystis*. *Botryococcus* form irregular clusters of cells, which might be more easily trapped by the midge larvae. The reason for low feeding preference of larvae on *S. quadricauda* is not clear.

Cyanobacteria were generally digested more efficiently than Chlorophyta by *C. crassicaudatus* larvae (Fig. 1). Among cyanobacteria, *A. flos-aquae* and *L. cf. aeruginosa* were digested significantly more efficiently than *Microcystis* species. Among Chlorophyta, *S. quadricauda* was found to be digested more efficiently than *B. braunii*. The latter species appeared to remain undigested by *C. crassicaudatus* larvae (Fig. 1). The algal food selection only partly corresponds with algal digestibility (Fig. 1); this finding is in agreement with observations

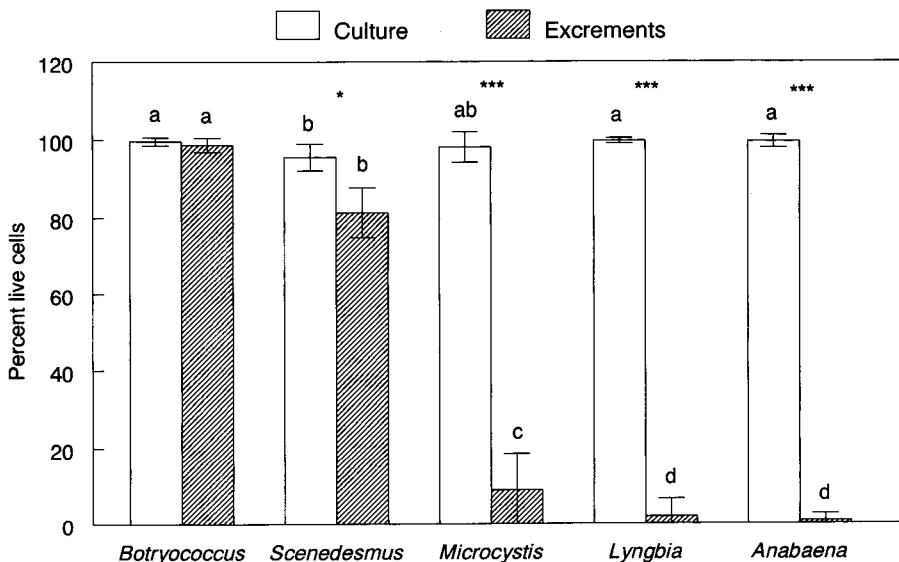


Fig. 1. Proportion of live cells in algal culture and in *Chironomus crassicaudatus* larval excrement. Statistically homogeneous groups of algal cultures or excrement are marked by the same letter (ANOVA, Shefe, $P < 0.05$). Significant differences between culture and excrement of the same algal species (t -test) are * $P < 0.05$ and *** $P < 0.001$.

of Hubert and Lukešová (2001) on algal selection and digestion by terrestrial mites.

Excrement cultures revealed that for all algal species, some cells were able to survive and reproduce in appropriate media. For all algal species except *L. cf. aeruginosa*, viable algal cultures were produced from all 5 excrements. In the case of *L. cf. aeruginosa*, 4 of 5 excrements produced viable algal cultures. The differences between individual algal species were not significant (Kruskal-Wallis test).

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